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Direct potentiometric quantification of histamine using solid-phase imprinted nanoparticles as recognition elements

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ABSTRACT

A new potentiometric sensor based on molecularly imprinted nanoparticles produced via the solid-phase imprinting method was developed. For histamine quantification, the nanoparticles were incorporated within a membrane, which was then used to fabricate an ion-selective electrode. The use of nanoparticles with high affinity and specificity allowed for label-free detection/quantification of histamine in real samples with short response times. The sensor could selectively quantify histamine in presence of other biogenic amines in real wine and fish matrices. The limit of detection achieved was 1.12×10^{-6} mol L^{-1}, with a linear range between 10^{-6} and 10^{-2} mol L^{-1} and a response time below 20 s, making the sensor as developed a promising tool for direct quantification of histamine in the food industry.

1. Introduction

Histamine is an undesirable biogenic amine (BA) whose presence has been reported in foods (Al Bulushi et al., 2009; Naila et al., 2010) like fish and fish products, cheese and fermented food or alcoholic beverages like wine (Beneduce et al., 2010; Lonvaud-Funel, 2001) and beer (Tang et al., 2009). It originates as a consequence of histidine decarboxylation by microorganisms, through decarboxylase enzymes, its presence leading to reduced foodstuff quality.

Malolactic fermentation is one of the main processes contributing to the presence of BA in wines, however, the level of several BA can subsequently increase during ageing (Garcia-Marino et al., 2010). Histamine in particular, is responsible for wine intolerance (Konakovsky et al., 2011). Levels of this compound have been reported in wines from different countries (Konakovsky et al., 2011; Marcobal et al., 2005; Martuscelli et al., 2013; Pineda et al., 2011), different grape varieties (Hernandez-Orte et al., 2008; Martinez-Pinilla et al., 2013) or different ageing times (Hernandez-Orte et al., 2008; Martinez-Pinilla et al., 2013). Results range from 1 to 20 mg L^{-1} (9×10^{-6} to 1.8×10^{-4} mol L^{-1}) even if reported values are mostly below 5 mg L^{-1} (4.5×10^{-5} mol L^{-1}). Negative effects appear on consumption of beverages having more than 8 mg L^{-1} (Beneduce et al., 2010).

Concerning seafood products, the BA content is time–temperature dependent and can be controlled by preventing time–temperature excess. However it is not always possible to control BA production since some bacteria are capable of producing BA below 5 °C (Naila et al., 2010). Tuna and mackerel, which belong to the *Scombridae* family, possess highest histidine levels in their muscle tissue, and as consequence, higher histamine levels are found in products containing these species; its consumption can lead to histamine poisoning, also known as scombroid poisoning (Hungerford, 2010) when levels of BA, particularly histamine, are above 500 mg kg^{-1} (Naila et al., 2012). In humans, 8–40 mg histamine can cause slight poisoning, over 40 mg moderate and over 100 mg severe poisoning (Önal, 2007; Shalaby, 1996). Accordingly, the European Council Directive 91/493/CEE established that mean analysis level must not exceed 100 mg kg^{-1} histamine in fish products.

Histamine and BA control in foods requires reliable analytical methods capable of accurate determination, for toxicity reasons and for the possibility to use BA levels as an indicator of food quality/freshness (Önal, 2007). In this sense, chromatographic methods have gained advantage over others such as capillary electrophoresis or electroanalytical methods (Önal, 2007). HPLC coupled to UV, DAD and fluorescence detectors or coupled to a mass spectrometer (Millan et al., 2007) have commonly been employed for BA quantification (Önal et al., 2013). However, the methods mentioned above all require sample processing and complex equipment which is usually laboratory based. For the purposes of process monitoring and quality control of raw materials in the food industry, a portable sensor capable of instant and label-free determination of histamine is

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highly desirable. Few electrochemical sensors have been described so far, probably due to the high oxidation potential of histamine (1.2 V approx.) and the consequent high background current and signal noise (Yang et al., 2013). Voltammetric multisensor (Rodriguez-Mendez et al., 2009), potentiometric sensors based on crown ether (Elmosallamy, 2012) or porphyrins (Amini et al., 1999) as carriers in poly(vinylchloride) (PVC) membranes constitute some of the few published examples. These types of membranes are comprised of a carrier immobilised in a matrix made of a PVC and plasticizer mixture(Javanbakht and Akbari-adergani, 2012; Prasada Rao and Kala, 2008). Most commonly employed plasticizers are phthalate derivatives such as dibutyl, dioctyl and bis(2-ethylhexyl) phthalate (Arvand and Alirezanejad, 2011; Moreira et al., 2010; Sadeghi et al., 2011), sebacate derivatives such as bis(2-ethylhexyl) sebacate (Alizadeh et al., 2012) or other compounds such as 2-nitrophenyl octyl ether (Kamel et al., 2012), being the latter the most widely employed.

Molecular imprinting technology (Chen et al., 2011; Li et al., 2011; Spivak, 2008) can provide robust and stable synthetic molecular receptors in order to overcome the problems of stability associated with biosensors. Molecularly imprinted polymers (MIP) in bulk/microparticle format have been successfully applied to sample pre-treatment (Gomez-Caballero et al., 2013) including extraction and quantification of histamine (Basozabal et al., 2013). This type of synthetic receptors can in theory be adapted to manufacture robust histamine sensors allowing selective, real-time quantification. MIPs however are perceived to have several disadvantages. Among these are heterogeneous distributions of binding sites, responsible for high levels of non-specific binding, difficult integration with sensors and labour-intensive procedures for their synthesis. In order to address these problems, a new method, easy to implement and automate was recently developed (Poma et al., 2013) and utilised to produce the molecularly imprinted nanoparticles (MIN) as used here for sensor development. Unlike traditional protocols, where the template is free in solution, this methodology relies on template immobilised on a solid-phase and on subsequent affinity-based purification of the imprinted particles, accordingly, this method is called solid-phase imprinting. MIN with high affinity/specificity and a monodisperse distribution of binding site affinities can be reliably produced this way and in a short time period (Ambrosini et al., 2013; Moczko et al., 2013a, 2013b; Poma et al., 2013). Additionally, due to the affinity purification step used during synthesis, the amount of high-affinity binding sites in the final product (MIN) is maximised, as polymer particles with low or no affinity are removed, leading to reduced non-specific interactions when the product is in use, like in sensor applications.

Here we describe the integration of anti-histamine MIN with a potentiometric transducer for rapid and label-free quantification of histamine in fish and wine samples with minimal sample pre-treatment. The sensor was fabricated by incorporation of MIN in PVC membranes. The developed potentiometric sensor showed good selectivity and was capable of reproducible and specific detection and quantification of histamine in wine and fish matrices.

2. Materials and methods

2.1. Materials

Acetonitrile (ACN), ethanol, acetone, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), trimethylolpropane trimethacrylate (TRIM), pentaerythritol-tetrakis-(3-mercaptopropionate) (PETMP), phosphate buffer solution (PBS) tablets (pH 7.4), sodium hydroxide, glutaraldehyde (GA), histamine, histidine, *N*-[3-(trimethoxysilyl)propyl]ethylenediamine (TPED), sulphuric acid, hydrogen peroxide were purchased from Sigma-Aldrich, UK. *N*,*N*-diethyldithiocarbamic acid benzyl ester (DABE) was obtained from TCI Europe (Belgium). Solid glass beads (65 μm < diameter-< 106 μm, average 90 μm) purchased from Blagden Chemicals, UK. Deionised water was obtained from a Millipore (MilliQ) purification system. Sensor membrane components poly(vinyl chloride) (PVC), 2-nitrophenyl octyl ether (NPOE), dibutyl phthalate (DBP), dioctyl phthalate (DOP) and potassium tetrakis (4-chlorophenyl)borate (kTpBCl), hydrochloride salts of biogenic amines (histamine, tryptamine, tyramine, 2- phenylethylamine, putrescine and cadaverine) and the amino acids L-trypthophan, L-histidine and L-phenylalanine were from Sigma-Aldrich, Spain. All chemicals were analytical or HPLC grade and used as received.

2.2. Synthesis of histamine molecularly imprinted nanoparticles

The protocol for the solid-phase synthesis with immobilised histamine template was adapted from (Moczko et al., 2013a). The composition of the polymerisation mixture was previously optimised (Guerreiro et al., 2009; Poma et al., 2013). Monomer mixture was prepared by mixing MAA (1.44 g) as functional monomer, EGDMA (1.62 g) and TRIM (1.62 g) as cross-linkers, DABE (0.37 g) as iniferter (initiator) and PETMP (0.09 g) as chain transfer agent dissolved in ACN (5.26 g). The mixture was then bubbled with N_2 for 10 min to remove dissolved oxygen. Histamine-derivatised glass beads (solid phase, 25 g), prepared as described in supplementary information, were placed in a 200 mL flat-bottomed glass beaker (with a flat glass cover) and degassed in vacuo for 20 min, then the atmosphere inside the beaker replaced with N_2. The polymerisation mixture was poured onto the solid phase (under a N_2 stream) and the vessel then placed between two UV light sources (one on top and one below the beaker) Philips model HB/171/A, each fitted with 4×15 W lamps, for 2.5 min. After polymerisation, the contents of the beaker were transferred into an SPE cartridge fitted with a polyethylene frit (20 μm porosity) in order to perform the temperature-based affinity separation of MIN. The temperature of ACN and the SPE cartridge was kept between 20 and 22 °C. Washing was performed with 8 bed volumes of ACN (relative to the volume of the solid phase, approx. 20 mL). This was done in order to remove non polymerised monomers and low affinity polymer. The effectiveness of the washing was verified by measuring UV absorbance of washing aliquots. After washing at low temperature, SPE cartridge containing the glass beads and the attached MIN was conditioned at 60 °C and washed with hot ACN at 60 °C (5 bed volumes) in order to elute the high-affinity nanoparticles. The total volume of the collected solution of MIN in ACN was 100 mL.

2.3. Surface plasmon resonance (SPR) analysis of MIN

SPR experiments were performed on a Biacore 3000 SPR system (GE Healthcare Life Sciences, UK). Au-coated chips (SIA Kit Au, GE Healthcare Life Sciences), were cleaned in piranha solution (H_2SO_4/H_2O_2, 3:1 v/v) for 5 min, and rinsed with water. For the immobilisation of histamine or histidine, the following procedure was used: cleaned chips were dried under N_2 and placed in a solution of 2% TPED/toluene overnight. After rinsing with acetone, the gold chip surface was placed in 5% GA solution in PBS during 2 h, rinsed with water and then incubated with 0.5 mg mL^{-1} solution of either histamine or histidine in PBS overnight then washed with water. The chips were assembled on their holders and stored under Ar at 4 °C. Prior to SPR analysis, 1 mL MIN were transferred from ACN to PBS, by diluting solutions 1:10 in PBS, then concentrated down to 1.5 mL using centrifugation cartridges (Amicon, ultracel membrane, 30 kDa MWCO, 15 mL,